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INVESTOR IN PEOPLE

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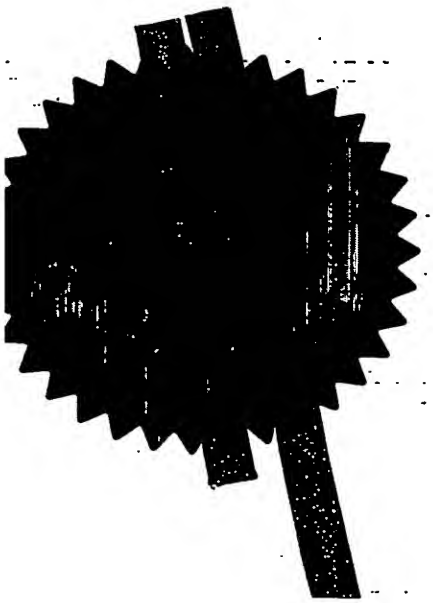

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# Request for grant of a patent

**1/77**

**The Patent Office**

Cardiff Road
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28 NOV 2003

| | | |
|---|---|---|
| 1. | Your reference | SVH/45058GB1 |
| 2. | Patent application number | 0327723.3 |
| 3. | Full name, address and post code of the or each applicant | Vectura Ltd<br>1 Prospect West<br>Chippenham<br>SN14 6FH<br>Wiltshire |
| | Patents ADP number | 08610727001 |
| | If the applicant is a corporate body, give the country/state of its incorporation | United Kingdom |
| 4. | Title of the invention | Pharmaceutical Compositions |
| 5. | Name of your agent | VENNER, SHIPLEY & CO |
| | "Address for service" in the United Kingdom to which all correspondence should be sent | 20 LITTLE BRITAIN<br>LONDON<br>EC1A 7DH |
| | Patents ADP | 1669004 ✓ |

| 6. | If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or each of these earlier applications and the or each application number | Country | Priority application number | Date of filing |
|---|---|---|---|---|
| | | United Kingdom | 0321611.6 | 15 Sept 2003 |

| 7. | If this application is divided or otherwise derived from an earlier UK application, give the number and filing date of the earlier application | Number of earlier application | Date of Filing |
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**Patents Form 1/77**

8. Is a statement of inventorship and of right to grant of a patent required in support of this request? *(Answer 'YES' if:*
*a) any applicant in 3. above is not an inventor, or*
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*c) any named applicant is a corporate body)*

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| | |
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| Continuation sheets of this form | |
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| Claim(s) | 3 |
| Abstract | 1 |
| Drawing(s) | |

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| Priority documents | |
| Translations of priority documents | |
| Statement of inventorship and right to grant of a patent *(Patents Form 7/77)* | |
| Request for preliminary examination and search *(Patents Form 9/77)* | |
| Request for substantive examination *(Patents Form 10/77)* | |
| Any other documents | |

11. I/We request the grant of a patent on the basis of this application.

Signature: James Skyrley & Co.

Date: 28 November 2003

12. Name and daytime telephone number of person to contact in the United Kingdom

Siân Hughes
020 7600 4212

# Pharmaceutical Compositions

The present invention relates to pharmaceutical compositions which are useful in the treatment of diseases where excess mucus is present in the respiratory tract, such as cystic fibrosis and chronic obstructive pulmonary disease. In particular, the invention relates to pharmaceutical compositions for administration by pulmonary inhalation.

Mucus is a viscous gel, the properties of which are dependent on a variety of factors. Mucus is primarily made up of a mixture of variable amounts of mucous glycoproteins, water, low molecular weight ions, proteins and lipids. These components interact in a number of ways and these interactions create the three-dimensional structure of the gel and determine the gel's viscosity and elasticity.

Mucin is the principle polymeric component of the mucus gel and consists of a peptide backbone with glycosylated and non-glycosylated domains and oligosaccharide chains. The presence of sulphated and sialic terminals makes the molecule highly polyanionic. The mucins form a polydisperse group of densely charged linear polymers, some of which are up to 6μm in length, with random tangles. The rheological properties are mainly dependent on the tangle density, which in turn is determined by the degree of mucus hydration and mucin molecule length. The necrotic activated neutrophils release large amounts of DNA, actin and proteins which also polymerise and interact with mucin. This process considerably increases the tangle density to form highly viscoelastic mucus gels.

A variety of different types of bonds within airway mucus affect the chemical and physical properties of the mucus, such as viscoelasticity. Disulphide bonds are covalent bonds which link glycoprotein subunits into the large, extended macromolecular chains known as mucins. Cross-links form between adjacent mucin polymers, probably as a result of their large size. The sugar units, which make up the oligosaccharide side-chains and account for about 80% of the mucin weight, form hydrogen bonds with complimentary units on neighbouring mucins. Although each individual bond is weak and readily dissociates, there are very large numbers of

bond sites, making this a significant type of bonding within the mucus. In addition, mucins are also ionized, containing both positively charged amino acid residues as well as negatively charged sugar units, principally sialic acid and sulphated residues. The degree of mucin ionisation may actually increase in airway disease. For example, in cystic fibrosis (CF) the proportion of sulphated residues is further elevated because of alterations in glycosyl transferase activities within the Golgi apparatus. The ionic interactions between fixed negative charges result in a stiffer, more extended macromolecular conformation, effectively increasing the polymer size and adding to the numbers of entanglements. Finally, in airway diseases characterized by infection and inflammation, such as CF, high molecular weight DNA and actin filaments are released by dying leukocytes, and exopolysaccharides are secreted by bacteria. These add further bonding and bulk to the mucus.

Mucus is a critical component of the primary defence mechanism of the respiratory tract, trapping inhaled particulate and microbial material for removal via the mucociliary system. However, when this mechanism fails to clear sufficiently, mucus accumulates and must be coughed up as sputum, otherwise it is retained in the respiratory tract and can encourage the colonisation by microorganisms which may lead to chronic lung inflammation and obstruction.

Retention of the mucus in the respiratory tract presents a particular problem as it not only obstructs the airways but also facilitates infection and promotes a self-perpetuating cycle of infection and inflammation. Pathological agents such as bacteria (e.g. Pseudomonas aeruginosa) are often able to establish colonies within the mucus.

Problems tend to arise when the initial bacterial infection stimulates neutrophil chemotaxis, but the neutrophils are unable to effectively clear. Defective neutrophil apoptosis and impaired phagocytosis are key factors in the pathogenesis of lung disease in CF. Neutrophil proteases and oxidants are released during the process and these have a number of effects. They cause both cellular damage and impairment of ciliary movement. They are also potent secretagogues and actually enhance further mucus secretion. The proteases also cleave anti-proteases and cell

surface markers, further impairing the host defence mechanisms. Thus, the cycle is perpetuated as these effects further impair mucus clearance at the same time as increasing mucus secretion, encouraging bacterial stasis and promoting airway inflammation. Therefore, the failure of the neutrophils to clear the original infection actually leads to a rapid deterioration of the situation and the process accounts for much of the morbidity and mortality observed in patients with CF.

There are two main causes of mucus retention. The first is airway mucus hypersecretion, where the body produces and secretes elevated levels of mucus and the mucociliary system is unable to cope with and clear the large amounts of mucus quickly enough. The second cause is where the mucus has abnormal viscoelasticity. Where the mucus has an unusually high viscoelasticity, it is much more difficult for the mucociliary system to move the mucus and clear it from the airways.

Classical courses of action taken to treat individuals afflicted with airway hypersecretion and/or abnormal mucus viscoelasticity include antibiotic therapy, administration of bronchiodilators, use of systemic or inhaled corticosteroids, or oral administration of expectorants for liquefaction of the mucus. It is also known to treat the sufferers with aerosol delivered "mucolytic" agents, such as water and hypertonic saline solution. Recombinant human DNase I (rhDNase) has been used to treat CF sufferers. The rhDNase is thought to enzymatically digest the naked DNA released into the airway surface fluid from bacteria, neutrophils, and other cellular debris. It is this DNA which is thought to contribute to the elevated viscoelasticity of the mucus in CF sufferers.

However, these conventional approaches have met with only limited success and there is the need for cheap and effective treatment for mucus retention in the lungs. What is more, it is an aim of the present invention to provide a treatment which will lead to a reduction in the mucus elasticity and viscosity and which will result in improved cough and airway clearance of the mucus and also enable clearance by means of ciliary action.

Agents such as rhDNase, which digests the naked DNA in the mucus, and gelsolin, which digests actin in the mucus, have been shown to affect the elasticity components of the network, as opposed to the viscosity. In model studies, this will tend to improve cough and airway clearance, rather than helping clearance by means of ciliary action.

It has been suggested that agents which disrupt the cross-links in the mucus cause a reduction in both elasticity and viscosity. This is the preferred result, as it will lead to an improvement in ciliary clearance, according to model studies.

Dextrans have been identified as being a potentially useful agent for improving mucus clearance in International Publication No. WO 99/01141. In this patent application, it is suggested, from *in vitro* models, that dextrans decrease mucus viscoelasticity and increase mucociliary clearability. The dextrans are thought to have this effect by disrupting the hydrogen bonding between mucins within the three-dimensional mucus structure. It is hypothesised that the dextrans compete with the mucin for the hydrogen bonding sites, resulting in the substitution, by dextran carbohydrate moieties, of oligosaccharide moieties linked to high molecular weight mucin peptides that make up the mucus gel. The dextrans used have significantly lower molecular weight and so these new hydrogen bonds are structurally and rheologically ineffective, thus reducing the overall cross-link density within the mucus and this, it is believed, improves mucus clearance by ciliary and cough mechanisms.

In a later patent application, International Publication No. WO 01/15672, it is further suggested that the action of dextrans may be further enhanced by using charged forms. A charged dextran, for example dextran sulphate, is thought to have dual activity. Firstly, it is said to have the effects due to competition for hydrogen bonding sites as discussed above. Secondly, the ionic nature of the charged dextran is thought to have an additional effect, shielding some of the fixed charges along the macromolecular core of the mucin polymer, making it less stiff and reducing the number of entanglement cross-links with neighbouring macromolecules within the mucus and thereby reducing viscoelasticity due to ionic interactions.

In WO 01/15672, it is also suggested that the charged oligosaccharide heparin is not suitable for treating pulmonary diseases such as CF, because it is expensive to produce and, more significantly, because it could potentially have toxic side-effects such as pulmonary hemoptysis, which is bleeding of the tracheobronchial mucosa.

Heparin is a linear polysaccharide which, along with related proteoglycans such as heparan sulphate, is a member of the group of macromolecules referred to as glycosaminoglycans. Owing to their linear anionic polyelectrolyte structure, these macromolecules are involved in various biological processes. While heparin has been used largely for its anticoagulant effects based on its binding to plasma anti-thrombin III, there is evidence that heparin and other glycosaminoglycans also possess various anti-inflammatory and immunoregulatory properties, including the modulation of T-lymphocytes, complement activation, inhibition of neutrophil chemotaxis, smooth muscle growth and reduction of intrinsic DNA viscosity.

Heparin is a heterogeneous mixture of variably sulphated polysaccharide chains with a molecular weight range of 6000 to 30,000 Daltons. Whole or unfractionated heparin (UFH) may be fractionated to give low and high molecular weight fractions, as is well known in the art. Fractionated, low molecular weight heparin (LMWH) has been shown to reduce the viscoelasticity of dog mucus and improve mucociliary clearance on a frog palate model.

The effects of inhaling an aqueous solution of heparin using a nebuliser on bronchial asthma have been the subject of several studies. However, the results of these studies have been inconsistent, possibly because of the difficulty in quantifying the dosages of inhaled heparin reaching the lower respiratory tract.

Whilst the prior art discusses the possibility of combining hydrogen bond competition and ionic shielding in order to provide a two-fold mode of reducing mucus viscoelasticity, there are even further mechanisms by which the viscoelasticity may be reduced and the present invention seeks to use these other mechanisms to provide an even more efficient means for assisting mucus clearance,

especially in patients suffering from conditions such as cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), chronic bronchitis, acute asthma, or bronchiectasis.

Mucus clearance can be improved by reducing the viscosity and elasticity of the mucus gel. There are a number of mechanisms by which these properties can be affected to assist clearance, by coughing, by ciliary movement or a combination of the two.

Firstly, the cross-links within the mucus gel structure can be disrupted. This can be achieved by agents which break the di-sulphide bonds between the glycoproteins within the mucus. Alternatively or additionally, the cross-links within the mucus gel structure may be disrupted by agents which compete for hydrogen bonding sites, as described above in relation to dextran. Furthermore, the ionic bonding which exists within the gel can also be disrupted, by shielding the charges using an ionic agent. This has been described in connection with the use of a charged dextran.

Secondly, the mucus may be diluted by increasing its water content. This will reduce the gel's viscosity and will ease mucus clearance. This may be done by administering an agent to the mucus which will draw water into the mucus by exerting an osmotic effect. Alternatively, the water content of the mucus may be increased by agents which control sodium channels in the lung epithelium and are therefore able to block the uptake of salt and water across the airway epithelium.

Thirdly, digestion of the naked DNA and other cellular debris, such as filamentous actin, found in the mucus will also reduce the viscosity and elasticity of the mucus.

In a first aspect of the present invention, a composition for assisting mucus clearance is provided, the composition comprising one or more mucoactive agents for reducing cross-linking within the mucus; for diluting the mucus; and/or for digesting naked DNA and cell debris within the mucus. Preferably, the one or more mucoactive agents may also reduce inflammation.

Mucoactive agents are agents which affect the mucus in a way that assists clearance. Traditionally, such agents have been referred to as mucolytic agents, which may be inaccurate because the way in which they have their effect may not involve lysis.

In one embodiment of the invention, the mucoactive agent for reducing the cross-linking within the mucus is an agent which has hydrogen bonding sites which compete with the hydrogen bonding sites of the side-chains of the mucins which form hydrogen bonds with complimentary units on neighbouring mucins. Especially useful are charged mucoactive agents which, in addition to shielding some of the fixed charges along the macromolecular core of the mucin polymer also competing for the hydrogen bonding sites. This dual effect makes the mucus less stiff and reduces the number of entanglement cross-links with neighbouring macromolecules within the mucus, thereby reducing viscoelasticity due to ionic interactions.

In a particularly preferred embodiment of the present invention, the mucoactive agent for reducing cross-linking is a glycosaminoglycan. Glycosaminoglycans are a group of heteropolysaccharides which contain an N-acetylated hexosamine in a characteristic repeating disaccharide unit.

Heparin is a glycosaminoglycan and it is used for a variety of purposes. Heparin is often provided as a heterogeneous mixture of variably sulphated polysaccharide chains with a molecular weight range of 6,000 to 30,000 Daltons. Whole or unfractionated heparin (UFH) may be fractionated to give low and high molecular weight fractions, as is well known in the art.

In one embodiment of the present invention, the heparin used in the compositions comprises UFH, i.e. high molecular weight heparin. Surprisingly, it has been discovered that this high molecular weight form of heparin is effective in assisting mucus clearance and it has even been found to be more effective than low molecular weight fractions in reducing human mucus viscoelasticity *in vitro*.

In an alternative embodiment, the heparin used as a mucoactive agent in the compositions of the present invention is low molecular weight fractions of heparin.

Further, analogues of heparin are commercially available and may also be used as mucoactive agents in the present invention. Such analogues include sulphated heparin and glycosylated heparin. Surprisingly, the inventors have found that sulphated heparin is more effective that non-sulphated heparin in reducing the elasticity of human mucus. Accordingly, in a preferred embodiment of the present invention, the composition comprises sulphated heparin as a mucoactive agent.

Heparin derivatives are commonly termed heparinoids and these may also be used in the compositions of the present invention. Heparinoids are closely related to heparin and share many of its properties. Heparinoids are useful for reducing cross-linking in mucus and they also exhibit anti-inflammatory properties.

Chondroitins are another group of glycosaminoglycans which may be used in the present invention, and these include dermatan sulphate and chondroitin sulphates. Keratin sulphate and hyaluronic acid are further glycosaminoglycans which may be used as mucoactive agents in the compositions of the present invention, as are heparitin sulphates such as heparan sulphate proteoglycan.

In one embodiment of the present invention, the mucoactive agent is danaparoid sodium. This low molecular weight heparinoid contains a mixture of the sodium salts of heparan sulphate, dermatan sulphate and chondroitin sulphate and is useful in reducing the cross-linking in mucus. Another heparinoid which may be used comprises a combination of heparin, dermatan sulphate and chondroitin sulphate.

Naturally occurring and synthetic highly sulphated glucosaminoglycans are also examples of mucoactive agents which may be included in the compositions of the present invention. These compounds, which are also known as glycosaminoglycan polysulphate compounds, or sulfated mucopolysaccharides are also useful in reducing cross-linking within the mucus to be cleared.

Other polysaccharides aside from glycosaminoglycans may be used as mucoactive agents which reduce cross-linking within mucus, such as dextrans. Preferably, the polysaccharide mucoactive agent should have a relatively low molecular weight. For instance, the agent should have an average molecular weight of less than 30,000, more preferably less than 20,000 and even more preferably less than 10,000.

Finally, a further group of mucoactive agent capable of assisting mucus clearance are amino acids. Particularly effective amino acids include basic amino acids such as lysine, arginine and histidine, and their derivatives. These amino acids are thought to assist mucus clearance by increasing the transepithelial potential difference, causing a stimulation of chlorine transport, which induces water movemenmt into the epithelial lining fluid and further enhances the fluidification of the mucus. Other amino acids, such as cysteine is thought to disrupt the di-sulphide bonds in the mucus. Amino acids, including hydrophobic amino acids such as leucine also reduce cross-linking within the mucus.

In a further preferred embodiment, a charged agent is used to reduce cross-linking by shielding the charges on the mucins, thereby reducing the ionic interactions between adjacent mucins. Suitable charged agents include charged glycosaminoglycans, as discussed above, including, for example, heparin sulphate, heparan sulphates, or danaparoid sodium. Other polysaccharide sulphates or phosphates may also be used, such as dextran sulphates or phosphates. An alternative mucoactive agent capable of reducing cross-linking by shielding the charges on mucins is a sodium chloride solution or the like.

Suitable mucoactive agents for disrupting the cross-linking within the mucus by disrupting the di-sulphide bridges between glycoprotein subunits are compounds bearing free sulphydryl groups such as cysteine. These agents include the cysteine derivative N-acetylcysteine, the acetylcysteine salt derivative Nacystelyn (or NAL) and dithiothreitol.

In another embodiment of the present invention, the mucoactive agent for reducing cross-linking within the mucus is not dextran or a charged dextran, such as dextran

sulphate or dextran phosphate. In another embodiment, the agent for reducing cross-linking is a dextran having a molecular weight of more than 5,000.

In yet another embodiment of the present invention, the mucoactive agent for reducing cross-linking within the mucus is not heparin or heparin sulphate, or is not low molecular weight heparin.

Various mucoactive agents assist mucus clearance by increasing the water content of the mucus. Some of these agents act by drawing additional water into the mucus, and are often referred to as osmolar agents or even non-destructive mucolytics. Alternatively, these agents work by blocking the uptake of salt and water across airway epithelium.

Suitable mucoactive for inclusion in the compositions of the present invention which act by drawing water into the mucus include low molecular weight sugars such as dextrans, dextrin, mannitol, glucose or urea. Various other monosaccharides, disaccharides and oligosaccharides also have an osmolytic effect. Amiloride is an agent which is supposed to block the uptake of salt and water across airway epithelium, thereby increasing hydration and diluting the macromolecular components of the mucus. Some of the derivatives of amiloride have a similar activity, including phenamil and benzamil.

Examples of mucoactive agents which are capable of assisting mucus clearance by digesting naked DNA and cell debris within the mucus include rhDNase, which digests the naked DNA. Filamentous actin may be degraded by depolymerising agents such as gelsolin and thymosin ß4.

Mucoactive agents which reduce inflammation include the glycosaminoglycans discussed above, and in particular heparin, the heparinoids and the chondroitins. The use of such mucoactive agents allows the compositions of the present invention to simultaneously attack the excess mucus in the airways, but also to alleviate one of the particularly unpleasant results of that excess mucus, namely inflammation, which

often results from the infection which is effectively encouraged by the excess mucus, as discussed above.

As will be clear from the foregoing discussion of mucoactive agents suitable for use in the present invention, many of these agents actually exhibit two or more of the desired effects on the mucus. For example, heparin reduces the cross-linking within the mucus and it has an anti-inflammatory effect. Dextrans may disrupt cross-linking in the mucus as well as triggering dilution of the mucus.

It should be noted that the heparin products such as unfractionated heparin include both high and low molecular weight heparin in a single product. These different forms of heparin may, as discussed above, have different effects on the mucus, so that combinations of hydrogen bond breaking, ionic interference and osmotic effect are observed from administration of this single product.

In one embodiment of the invention, the composition comprises a glycosaminoglycan and preferably a charged glycosaminoglycan.

In another embodiment of the present invention, the composition comprises at least two mucoactive agents. In one embodiment, at least one of the mucoactive agents is a glycosaminoglycan. In another embodiment the two or more mucoactive agents have different effects on the mucus to one another, as discussed above.

The combination of different types effects on the mucus, by virtue of the different mechanisms of assisting mucus clearance discussed above, is surprisingly effective. The combined effects are thought to reduce the viscosity and elasticity of the mucus, enabling clearance of the mucus from the lungs both through coughing and through ciliary movement.

What is more, some combinations of mucoactive agents exhibit a synergistic effect. For example, rhDNase has in the past been found to have limited effect on some patients and this was thought to be a result of the rhDNase having difficulty penetrating the mucus. However, when the rhDNase is co-administered with

another mucoactive agent which is capable of disrupting the cross-links within the mucus, for example heparin, the rhDNase is better able to penetrate the gel structure and is therefore more effective. Thus, the effect of the combination of mucoactive agents is greater than the sum of the effects of the agents when they are administered individually.

In a preferred embodiment of the invention, the composition includes a combination of an agent for reducing cross-linking and an agent for diluting the mucus. For example, the agent for reducing cross-linking may be heparin or heparin sulphate, while the agent for diluting the mucus may be a low molecular weight sugar such as dextran. Another combination comprises a mixture of different heparins or heprainoids. Alternatively, the combination may comprise an agent for reducing cross-linking, such as a glycosaminoglycan plus dextran, mannitol and/or lactose, in order to enhance the osmotic or hydrogen bond breaking effect. In another embodiment, the composition comprises an agent for reducing cross-linking, such as heparin, a heparinoid or other glycosaminoglycan and and amino acid such as lysine, cysteine or leucine. Another combination comprises heparin, dermatan sulphate and chondritin sulphate.

In another embodiment of the invention, the mucoactive agents are administered, either simultaneously or sequentially, with an antibiotic. For example, for treating CF, the antibiotic might be selected from tobramycin, gentamycin, ciprofloxin or colomycin. For treating COPD, the antibiotic might be amoxycillin, cotrimixazole or doxycycline. One or more antibiotics may be included in the composition with the one or more mucoactive agents.

In yet another embodiment of the invention, the mucoactive agents are administered, either simultaneously or sequentially, with a surfactant. Surfactants are known to reduce adherence of mucus and help it to be cleared or may aid the spreading of the formulation once it is in the lungs. Surfactants, such as lecithin, may advantageously affect the surface tension of the mucus and therefore assist its clearance.

The doses of mucoactive agents required to have the desired effect of assisting mucus clearance will clearly depend upon the agents used. However, in the case of glycosaminoglycans such as heparin and heparinoids, the preferred delivered dose is preferably 1-200mg, 50-120mg, 5-80mg, 10-40mg or 2-10mg.

These are relatively large doses, even at the lower ends of the ranges and this presents some delivery problems which are addressed below.

The compositions of the present invention are well suited to the treatment of pulmonary and other diseases, whilst overcoming the problems associated with current treatments of such diseases. Preferably, the compositions would be used for treating diseases which have as a symptom the excess formation of mucus secretions in the airways, including chronic bronchitis, acute asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD), bronchiectasis, hypersecretion resulting from epithelial damage such as allergic stimuli or mechanical abrasions, and nasal hypersecretion.

In a particularly preferred embodiment of the first aspect of the invention, the composition for assisting mucus clearance is in the form of a dry powder.

There is a general prejudice in this technical field against treating the excess mucus in the lungs of a patient suffering from CF, COPD or the like with a dry powder formulation. Such conditions have, in the past, almost exclusively been treated with solutions. Despite this prejudice, it has been found that formulating the compositions comprising one or more mucoactive agents as a dry powder is linked with a number of significant advantages which enable the present invention to be put into practice and put into practice in a commercially attractive manner.

When solutions or suspensions are to be administered to the lung by inhalation, this is done using nebulizers. These devices dispense solutions and suspensions in the form of a fine spray, and they typically have a face mask attached, so that the subject may inhale the fine spray through the mouth or nose. However, nebulizers tend to be large devices and they are generally not portable, frequently because they

are pressurised by an oxygen tank. For this reason, nebulizers tend to be used to dispense medicaments to immobile patients and they are often unsuitable where an easy and convenient (self-)administration of a medicament is desired, as in the case with the present invention.

A further problem associated with the use of nebulizers is the difficulty obtaining accurate information regarding the dose actually delivered to the patient. There is also a general lack of precision, reproducibility and efficiency in the delivery of the medicament, leading to the need to increase the dose administered which results in drug wastage and an increased risk of adverse effects.

Finally, where relatively large doses of an active agent are to be administered to a patient, this often requires inhalation of the fine spray over an extended period of time. For example, in one study of the effect of inhaled heparin, a relatively small dose of 8,000IU of heparin had to be administered over a period of 15 minutes. This is clearly not a convenient mode of administration.

In contrast, the devices used to deliver dry powder formulations are simple and relatively cheap, so that they can even be disposable. Furthermore, the devices are small and therefore easily portable. They are also very easy for a patient to use.

However, there are problems associated with formulating the compositions of the present invention as a dry powder. Firstly, due to their polyanionic nature, glycosaminoglycans are "sticky" molecules and they have been found to readily form aggregates when provided in particulate formation. Such aggregates are too large to reach the deep lung upon inhalation. Secondly, in order to assist in the clearance of mucus from the airways, a large dose of mucoactive agent is required. In order to be able to administer a dose of the order of 10's of milligrams of active agent by dry powder inhalation, a high dosing efficiency is required otherwise an unacceptable amount of powder would have to be inhaled into the lungs. Dry powder inhalers which are currently commercially available tend to have relatively poor dosing efficiency. With many of these dry powder formulations, it has been found that

frequently only a small amount (often only about 10%) of the active particles that leave the device on inhalation are deposited in the lower lung.

A high efficiency administration of a dry powder would mean that the amount of mucoactive agent delivered to the patient is predicable, allowing the variation between doses to be minimised. This in turn means that the amount of agent administered can be adjusted to be much closer to the minimum required in order to achieve the desired therapeutic effect.

The present invention provides methods and formulations which enable the compositions of mucoactive agents to be efficiently dispensed as dry powders. These aspects of the invention are discussed in greater detail below.

Formulating dry powder formulations for use in the present invention presents problems, especially where the composition includes a "sticky" glycosaminoglycan such as heparin or heparinoids. The nature of these compounds mean that they do not lend themselves well to formulation in fine particulate form. Therefore, it is necessary to employ special formulating techniques in order to produce a powder which can be dispensed in an efficient manner so that it can assist mucus clearance. If a simple dry powder formulation is used, the dosing efficiency will be such that it will be all but impossible to administer enough of the mucoactive agent or agents to the lungs to have the desired effect of assisting mucus clearance.

The dosing efficiency is highly dependent on the fine particle fraction (FPF) of the dry powder formulation and various excipients need to be added in order to ensure that a high enough FPF is achieved.

A further obstacle to being able to deliver the composition of the present invention as a dry powder is the high dose of the mucoactive agents required in order to have an effect. The only way that a high enough dose can be administered without exposing the lungs to too much dry powder is for the dosing efficiency to be high. The usual maximum dose of drug delivered using a dry powder is in the order of 5mg. In the present invention, the doses will frequently far exceed that level and,

unless the dosing efficiency is very high, it will simply not be possible to deliver the large doses of mucoactive agent required.

Thus, the present invention is not merely the decision to use certain mucoactive agents or combinations of these agents. Rather, there is a significant amount of work required to put the invention into practice in such a way that it could be a pharmaceutical product.

The composition according to the present invention may be dispensed using any device which is suitable for pulmonary administration of a dry powder. Preferably, the composition is suitable for administration using a dry powder inhaler (DPI).

The compositions of the present invention may also include other substances, such as stabilisers or excipient materials. The particles of mucoactive agent will usually comprise at least 1% mucoactive agent, at least 50%, at least 75%, at least 90%, at least 95%, or at least 99% mucoactive agent. The particles of mucoactive agent may also include other substances such as stabilisers or excipient materials.

Other particles or materials included in the composition are intended to assist the efficient and reproducible delivery of the active particles from the delivery device to the lower respiratory tract or deep lung and these will be discussed in detail below.

The delivery of dry powder pharmaceutical compositions to the respiratory tract is known to present certain problems. The inhaler device (usually a DPI) should deliver the maximum possible proportion of the active particles expelled to the lungs, including a significant proportion to the lower lung, preferably at the low inhalation capabilities to which some patients, especially asthmatics, are limited. As a result, much work has been done on improving dry powder formulations to increase the proportion of the active particles which is delivered to the lower respiratory tract or deep lung.

The type of dry powder inhaler used will affect the efficiency of delivery of the active particles to the respiratory tract. Also, the physical properties of the powder

affect both the efficiency and reproducibility of delivery of the active particles and the site of deposition in the respiratory tract.

On exit from the inhaler device, the active particles should form a physically and chemically stable aerocolloid which remains in suspension until it reaches a conducting bronchiole or smaller branching of the pulmonary tree or other absorption site, preferably in the lower lung. Preferably, no active particles are exhaled from the absorption site.

When delivering a formulation to the lung for local action, the size of the active particles within the formulation is very important in determining the site of the absorption in the body.

For formulations to reach the deep lung via inhalation, the active agent in the formulation must be in the form of particles (active particles) that are very fine, for example having a mass median aerodynamic diameter (MMAD) of less than 10μm. It is well established that particles having an MMAD of greater than 10μm are likely to impact on the walls of the throat and generally do not reach the lung. Particles having an MMAD of 5 to 2μm will generally be deposited in the respiratory bronchioles whereas particles having an MMAD of 3 to 0.05μm are likely to be deposited in the alveoli or be absorbed into the bloodstream.

As the mucoactive agents are to act directly on the mucus in the airways, the dry powder composition should be formulated for delivery to the lower respiratory tract. Thus, the dry powder formulation should preferably comprise particles of the mucoactive agent which have an MMAD of approximately 2-5μm. Preferably, at least 90% by weight of the mucoactive particles have a diameter within this range.

Due to the polyanionic nature of glycosaminoglycans, they readily form aggregates when provided in particulate formation. Such aggregates are too large to reach the deep lung. The inventors have, however, been able to provide particulate formulations comprising mucoactive agents such as glycosaminoglycans which are capable of being aerosolised in a dry powder inhaler and delivered to the deep lung.

Advantageously, the compositions of the present invention comprise at least 30%, at least 50%, at least 75%, at least 90%, at least 95% or at least 99% by weight of mucoactive agent based on the total weight of the formulation.

In addition to the "sticky" nature of mucoactive agents, the fine particles are also thermodynamically unstable due to their high surface area to volume ratio, which provides a significant excess surface free energy and encourages particles to agglomerate. In the inhaler, agglomeration of small particles and adherence of such particles to the walls of the inhaler are problems that result in the fine particles leaving the inhaler as large, stable agglomerates, or being unable to leave the inhaler and remaining adhered to the interior of the inhaler or even clogging or blocking the inhaler.

The uncertainty as to the extent of formation of stable agglomerates of the particles between each actuation of the inhaler and also between different inhalers and different batches of particles, leads to poor dose reproducibility. Furthermore, the formation of agglomerates means that the MMAD of the active particles can be vastly increased, so that the agglomerates of the active particles do not reach the desired part of the lung for the required therapeutic effect.

According to a preferred embodiment, the compositions of the present invention firstly provide a high fine particle fraction (FPF) and fine particle dose (FPD) upon aerosolisation of the formulation. Additionally, the compositions comprise particles of the correct MMAD to be deposited in the correct part of the lung. Advantageously, the present invention has identified a number of simple methods of preparing these compositions having good FPFs and FPDs and accurate particle size range.

The metered dose (MD) of a dry powder formulation is the total mass of active agent present in the metered form presented by the inhaler device in question. For example, the MD might be the mass of active agent present in a capsule for a Cyclohaler (trademark), or in a foil blister in an Aspirair (trademark) device.

The emitted dose (ED) is the total mass of the active agent emitted from the device following actuation. It does not include the material left on the internal or external surfaces of the device, or in the metering system including, for example, the capsule or blister. The ED is measured by collecting the total emitted mass from the device in an apparatus frequently identified as a dose uniformity sampling apparatus (DUSA), and recovering this by a validated quantitative wet chemical assay.

The fine particle dose (FPD) is the total mass of active agent which is emitted from the device following actuation which is present in an aerodynamic particle size smaller than a defined limit. This limit is generally taken to be 5μm if not expressly stated to be an alternative limit, such as 3μm or 2μm, etc. The FPD is measured using an impactor or impinger, such as a twin stage impinger (TSI), multi-stage liquid impinger (MSLI), Andersen Cascade Impactor or a Next Generation Impactor (NGI). Each impactor or impinger has a pre-determined aerodynamic particle size collection cut points for each stage. The FPD value is obtained by interpretation of the stage-by-stage active agent recovery quantified by a validated quantitative wet chemical assay where either a simple stage cut is used to determine FPD or a more complex mathematical interpolation of the stage-by-stage deposition is used.

The fine particle fraction (FPF) is normally defined as the FPD divided by the ED and expressed as a percentage. Herein, the FPF of ED is referred to as FPF(ED) and is calculated as FPF(ED) = (FPD/ED) x 100%.

The fine particle fraction (FPF) may also be defined as the FPD divided by the MD and expressed as a percentage. Herein, the FPF of MD is referred to as FPF(MD), and is calculated as FPF(MD) = (FPD/MD) x 100%.

The tendency of fine particles to agglomerate means that the FPF of a given dose is highly unpredictable and a variable proportion of the fine particles will be administered to the lung, or to the correct part of the lung, as a result.

In an attempt to improve this situation and to provide a consistent FPF and FPD, dry powder formulations often include additive material.

The additive material is intended to decrease the cohesion between particles in the dry powder formulation. It is thought that the additive material interferes with the weak bonding forces between the small particles, helping to keep the particles separated and reducing the adhesion of such particles to one another, to other particles in the formulation if present and to the internal surfaces of the inhaler device. Where agglomerates of particles are formed, the addition of particles of additive material decreases the stability of those agglomerates so that they are more likely to break up in the turbulent air stream created on actuation of the inhaler device, whereupon the particles are expelled from the device and inhaled. As the agglomerates break up, the active particles return to the form of small individual particles which are capable of reaching the lower lung.

In the prior art, dry powder formulations are discussed which include distinct particles of additive material (generally of a size comparable to that of the fine active particles). In some embodiments, the additive material may form either a continuous or a discontinuous coating on the active particles and/or any carrier particles.

Preferably, the additive material is an anti-adherent material and it will tend to reduce the cohesion between particles and will also prevent fine particles becoming attached to the inner surfaces of the inhaler device. Advantageously, the additive material is an anti-friction agent or glidant and will give better flow of the pharmaceutical composition in the inhaler. The additive materials used in this way may not necessarily be usually referred to as anti-adherents or anti-friction agents, but they will have the effect of decreasing the cohesion between the particles or improving the flow of the powder. The additive materials are often referred to as force control agents (FCAs) and they usually lead to better dose reproducibility and higher fine particle fractions.

Therefore, an FCA, as used herein, is an agent whose presence on the surface of a particle can modify the adhesive and cohesive surface forces experienced by that particle, in the presence of other particles. In general, its function is to reduce both the adhesive and cohesive forces.

In general, the optimum amount of additive material to be included in a dry powder formulation will depend on the chemical composition and other properties of the additive material and of the active material, as well as upon the nature of other particles such as carrier particles, if present. In general, the efficacy of the additive material is measured in terms of the fine particle fraction of the composition.

Known additive materials usually consist of physiologically acceptable material, although the additive material may not always reach the lung. For example, where the additive particles are attached to the surface of carrier particles, they will generally be deposited, along with those carrier particles, at the back of the throat of the user.

In a further attempt to improve this situation and to provide a consistent FPF and FPD, dry powder formulations often include coarse carrier particles of excipient material mixed with fine particles of active material. Rather that sticking to one another, the fine active particles tend to adhere to the surfaces of the coarse carrier particles whilst in the inhaler device, but are supposed to release and become dispersed upon actuation of the dispensing device and inhalation into the respiratory tract, to give a fine suspension. The carrier particles preferably have MMADs greater than 90μm.

The inclusion of coarse carrier particles is attractive where relatively small doses of active agent are dispensed. It is very difficult to accurately and reproducibly dispense very small quantities of powder and small variations in the amount of powder dispensed will mean large variations in the dose of active agent where the powder comprises mainly active particles. Therefore, the addition of a diluent, in the form of large excipient particles will make dosing more reproducible and accurate.

Carrier particles may be of any acceptable excipient material or combination of materials. For example, the carrier particles may be composed of one or more materials selected from sugar alcohols, polyols and crystalline sugars. Other suitable carriers include inorganic salts such as sodium chloride and calcium carbonate, organic salts such as sodium lactate and other organic compounds such as polysaccharides and oligosaccharides. Advantageously, the carrier particles are of a polyol. In particular, the carrier particles may be particles of crystalline sugar, for example mannitol, dextrose or lactose. Preferably, the carrier particles are of lactose or mannitol, which is a mucoactive agent, as discussed above.

Advantageously, substantially all (by weight) of the carrier particles have a diameter which lies between 20μm and 1000μm, more preferably 50μm and 1000μm. Preferably, the diameter of substantially all (by weight) of the carrier particles is less than 355μm and lies between 20μm and 250μm.

Preferably at least 90% by weight of the carrier particles have a diameter between from 60μm to 180μm. The relatively large diameter of the carrier particles improves the opportunity for other, smaller particles to become attached to the surfaces of the carrier particles and to provide good flow and entrainment characteristics and improved release of the active particles in the airways to increase deposition of the active particles in the lower lung.

The ratios in which the carrier particles (if present) and composite active particles are mixed will, of course, depend on the type of inhaler device used, the type of active particles used and the required dose. The carrier particles may be present in an amount of at least 50%, more preferably 70%, advantageously 90% and most preferably 95% based on the combined weight of the composite active particles and the carrier particles.

However, a further difficulty is encountered when adding coarse carrier particles to a composition of fine active particles and that difficulty is ensuring that the fine

particles detach from the surface of the large particles upon actuation of the delivery device.

The step of dispersing the active particles from other active particles and from carrier particles, if present, to form an aerosol of fine active particles for inhalation is significant in determining the proportion of the dose of active material which reaches the desired site of absorption in the lungs. In order to improve the efficiency of that dispersal it is known to include in the composition additive materials, including FCAs of the nature discussed above. Compositions comprising fine active particles and additive materials are disclosed in WO 97/03649 and WO 96/23485.

In light of the foregoing problems associated with known dry powder formulations, even when including additive material and/or carrier particles, it is an aim of the present invention to provide dry powder compositions which have physical and chemical properties which lead to an enhanced FPF and FPD. This leads to greater dosing efficiency, with a greater proportion of the dispensed active agent reaching the desired part of the lung for achieving the required therapeutic effect.

In particular, the present invention seeks to optimise the preparation of particles of active agent used in the dry powder composition by engineering the particles making up the dry powder composition and, in particular, by engineering the particles of active agent. Furthermore, cohesion between particles is to be reduced in order to enhance the FPF and FPD of the dry powder compositions. This is done by preparing the heparin particles in the presence of an FCA.

Whilst the FPF and FPD of a dry powder formulation are dependent on the nature of the powder itself, these values are also influenced by the type of inhaler used to dispense the powder. For example, the FPF obtained using a passive device will tend not to be as good as that obtained with the same powder but using an active device, such as an Aspirair (trade mark) device (see WO 01/00262 and GB 2353222).

In another embodiment of the invention, the dry powder composition has an FPF of at least 50%. Preferably, the FPF(ED) will be between 70 and 99%, more preferably between 80 and 99%.

In another embodiment, the FPF(MD) is at least 50%. Preferably, the FPF(MD) will be between 50 and 90%, more preferably between 60 and 70%.

In a yet another embodiment, the pharmaceutical composition comprises at least one mucoactive agent and a force control agent, the force control agent preferably being present on the surface of particles of mucoactive agent.

The preferred FCAs to be included in the compositions of the invention may be any of the additive materials discussed above. Preferably, the FCA is selected from amino acids, peptides and polypeptides having a molecular weight of between 0.25 and 1000 kDa and derivatives thereof, dipolar ions such as zwitterions, phospholipids such as lecithin, and metal stearates such as magnesium stearate. Particularly preferred are amino acids and especially leucine, lysine and cysteine, with leucine being the most preferred.

Known FCAs usually consist of physiologically acceptable material, although the FCA may not always reach the lung. For example, where the FCA particles are attached to the surface of carrier particles, they will generally be deposited, along with those carrier particles, at the back of the throat of the user.

Preferably, the FCAs used in the present invention are film-forming agents, fatty acids and their derivatives, lipids and lipid-like materials, and surfactants, especially solid surfactants.

Advantageously, the FCA includes one or more compounds selected from amino acids and derivatives thereof, and peptides and derivatives thereof. Amino acids, peptides and derivatives of peptides are physiologically acceptable and give acceptable release of the active particles on inhalation.

It is particularly advantageous for the FCA to comprise an amino acid. The FCA may comprise one or more of any of the following amino acids: leucine, isoleucine, lysine, cysteine, valine, methionine, and phenylalanine. The FCA may be a salt or a derivative of an amino acid, for example aspartame, acesulfame K, or acetyl cysteine. Preferably, the FCA consists substantially of an amino acid, more preferably of leucine, advantageously L-leucine. The D-and DL-forms may also be used. As indicated above, L-leucine has been found to give particularly efficient dispersal of the active particles on inhalation. Lysine and cysteine are also useful as FCAs. As discussed above, all of these amino acids are also mucoactive agents.

The FCA may include one or more water soluble substances. This helps absorption of the substance by the body if the FCA reaches the lower lung. The FCA may include dipolar ions, which may be zwitterions.

Alternatively, the FCA may comprise a phospholipid or a derivative thereof. Lecithin has been found to be a good material for use as an FCA.

The FCA may comprise a metal stearate, or a derivative thereof, for example, sodium stearyl fumarate or sodium stearyl lactylate. Advantageously, the FCA comprises a metal stearate. For example, zinc stearate, magnesium stearate, calcium stearate, sodium stearate or lithium stearate. Preferably, the FCA comprises magnesium stearate.

The FCA may include or consist of one or more surface active materials, in particular materials that are surface active in the solid state. These may be water soluble or able to form a suspension in water, for example lecithin, in particular soya lecithin, or substantially water insoluble, for example solid state fatty acids such as oleic acid, lauric acid, palmitic acid, stearic acid, erucic acid, behenic acid, or derivatives (such as esters and salts) thereof, such as glyceryl behenate. Specific examples of such materials are: phosphatidylcholines, phosphatidylethanolamines, phosphatidylglycerols, phosphatidylinositol and other examples of natural and synthetic lung surfactants; lauric acid and its salts, for example, sodium lauryl sulphate, magnesium lauryl sulphate; triglycerides such as Dynsan 118 and Cutina

HR; and sugar esters in general. Alternatively, the FCA may be cholesterol or natural cell membrane materials, including pollen or spore cell wall components such as sporo-pollenins.

Other possible FCAs include sodium benzoate, hydrogenated oils which are solid at room temperature, talc, titanium dioxide, aluminium dioxide, silicon dioxide and starch.

In embodiments, a plurality of different FCAs can be used.

Spray drying is a well-known and widely used technique for producing particles of material. To briefly summarise, the material to be made into particles is dissolved or dispersed in a liquid or can be made into a liquid which is sprayed through a nozzle under pressure to produce a mist or stream of fine droplets. These fine droplets are usually exposed to heat which evaporates the moisture almost simultaneously, leaving a dry powder.

According to another aspect of the present invention, the compositions of the present invention are prepared by spray drying. In one embodiment, the spray drying process involves co-spray drying the one or more mucoactive agents with one or more force control agents.

The combination or blend of one or more mucoactive agents and FCA which is spray dried to form a dry powder formulation can be a solution or suspension in a host liquid. In some embodiments, all or at least a proportion of the mucoactive agent and/or FCA is or are in solution in the host liquid before being subjected to spray drying. Substantially all of the mucoactive agent and FCA can be in solution in the host liquid before being subjected to spray drying.

The one or more mucoactive agents are preferably at least 1.5, 2, 4 and, more preferably, at least 10 times more soluble than the FCA in the host liquid at the spraying temperature and pressure. In preferred embodiments, this relationship exists at a temperature between 30 and 60°C and atmospheric pressure. In other

embodiments, this relationship exists at a temperature between 20 to 30°C and atmospheric pressure, or, preferably, at 20°C and atmospheric pressure.

In addition to the above discussed spray drying technique, alternative techniques for producing fine particles may be used, such as spray freeze drying and freeze drying.

In another embodiment of the invention, the one or more mucoactive agent are spray dried using a spray drier comprising a means for producing droplets moving at a controlled velocity and of a predetermined size. The advantages of this control of the droplet formation will be discussed in greater detail below.

Finally, the spray drying process may also include a further step wherein the moisture content of the spray dried particles is adjusted, in order to "fine-tune" the properties of the particles.

It has further been discovered that the FPF and FPD of the dry powder formulation is also affected by the means used to create the droplets which are spray dried. Different means of forming droplets can affect the size and size distribution of the droplets, as well as the velocity at which the droplets travel when formed and the gas flow around the droplets. In this regard, the velocity at which the droplets travel when formed and the gas (which is usually air) flow around the droplets can dramatically affect size, size distribution and shape of resulting dried particles.

A method of preparing a dry powder composition is provided, wherein the one or more mucoactive agents are spray dried using a spray drier comprising a means for producing droplets moving at a controlled velocity and of a predetermined droplet size. The velocity of the droplets is preferably controlled relative to the body of gas into which they are sprayed. This can be achieved by controlling the droplets' initial velocity and/or the velocity of the body of gas into which they are sprayed.

It is clearly desirable to be able to control the size of the droplet formed during the spray drying process and the droplet size will affect the size of the dried particle. Preferably, the droplet forming means also produces a relatively narrow droplet, and

therefore particle, size distribution. This will lead to a dry powder formulation with a more uniform particle size and thus a more predictable and consistent FPF and FPD.

The ability to control the velocity of the droplet also allows further control over the properties of the resulting particles. In particular, the gas speed around the droplet will affect the speed with which the droplet dries. In the case of droplets which are moving quickly, such as those formed using a 2-fluid nozzle arrangement (spraying into air), the air around the droplet is constantly being replaced. As the solvent evaporates from the droplet, the moisture enters the air around the droplet. If this moist air is constantly replaced by fresh, dry air, the rate of evaporation will be increased. In contrast, if the droplet is moving through the air slowly, the air around the droplet will not be replaced and the high humidity around the droplet will slow the rate of drying. As discussed below in greater detail, the rate at which a droplet dries affects various properties of the particles formed, including FPF and FPD.

Preferably the velocity of droplets at 10 mm from their point of generation is less than 100 m/s, more preferably less than 50 m/s, most preferably less than 20 m/s. Preferably the velocity of the gas, used in the generation of the droplets, at 10 mm from the point at which they are generated is less than 100 m/s, more preferably less than 50 m/s, most preferably less than 20 m/s. In an embodiment, the velocity of the droplets relative to the body of gas into which they are sprayed, at10 mm from their point of generation, is less than 100 m/s, more preferably less than 50 m/s, most preferably less than 20 m/s.

Preferably, the means for producing droplets moving at a controlled velocity and of a predetermined size is an alternative to the commonly used 2-fluid nozzle. In one embodiment, an ultrasonic nebuliser (USN) is used to form the droplets in the spray drying process.

Whilst ultrasonic nebulisers (USNs) are known, these are conventionally used in inhaler devices, for the direct inhalation of solutions containing drug, and they have

not previously been widely used in a spray drying apparatus. However, it has been discovered that the use of such a nebuliser in spray drying has a number of important advantages and these have not previously been recognised.

USNs use an ultrasonic transducer which is submerged in a liquid. The ultrasonic transducer (a piezoelectric crystal) vibrates at ultrasonic frequencies to produce the short wavelengths required for liquid atomisation. In one common form of USN, the base of the crystal is held such that the vibrations are transmitted from its surface to the nebuliser liquid, either directly or via a coupling liquid, which is usually water. When the ultrasonic vibrations are sufficiently intense, a fountain of liquid is formed at the surface of the liquid in the nebuliser chamber. Large droplets are emitted from the apex and a "fog" of small droplets is emitted. A schematic diagram showing how the USN works is shown in Figure 1.

The attractive characteristics of USNs for producing fine particle dry powders include: low spray velocity; the small amount of carrier gas required to operate the nebulisers; the small droplet size and narrow droplet size distribution produced; the simple nature of the USNs (the absence of moving parts which can wear, etc.); and the ability to accurately control the gas flow around the droplets, thereby controlling the rate of drying.

To elaborate, USNs do not separate the liquid into droplets by increasing the velocity of the liquid. Rather, the necessary energy is provided by the vibration caused by the ultrasonic nebuliser.

Thus, as an alternative to the conventional two-fluid nozzle, an ultrasonic nebuliser may be used to generate droplets of active agent, which are then dried within the drying chamber. In one arrangement, the USN is placed in the feed solution comprising an active agent in a specially designed glass chamber which allows introduction of the cloud of droplets generated by the USN directly into the heated drying chamber of the spray dryer.

In an embodiment of the present invention, the method of preparing the active particles involves the use of an ultrasonic nebuliser. Preferably, the ultrasonic nebuliser is incorporated in a spray drier.

Similar results to those shown above when using USNs are expected for spray drying using other means which produce low velocity droplets. For example, further alternative nozzles may be used, such as vibrating orifice nozzles. These nozzles, like the ultrasonic nozzles, are momentum free, resulting in a spray which can be easily directed by a carrier air stream.

Another attractive type of nozzle for use in a spray drying process is one which utilises electro-hydrodynamic atomisation. A tailor cone is created, for example, at a fine needle by applying high voltage at the tip. This shatters the droplets into an acceptable monodispersion. This method does not use a gas flow, except to transport the droplets after drying. An acceptable monodispersion can also be obtained utilising a spinning disc generator.

The nozzles such as ultrasonic nozzles, electrospray nozzles or vibrating orifice nozzles can be arranged in a multi nozzle array, in which many single nozzle orifices are arranged in a small area and facilitate a high total throughput of feed solution. The ultrasonic nozzle is an ultrasonic transducer (a piezoelectric crystal). If the ultrasonic transducer is located in an elongate vessel the output may be raised significantly.

When mucoactive particles are produced by spray drying, some moisture will remain in the particles. This is especially the case where the mucoactive agent is temperature sensitive and does not tolerate high temperatures for the extended period of time which would normally be required to remove further moisture from the particles.

The amount of moisture in the particles will affect various particle characteristics, such as density, porosity, flight characteristics, and the like.

A method of preparing a dry powder composition is also provided, wherein the method comprises a step of adjusting the moisture content of the particles.

In one embodiment, the moisture adjustment or profiling step involves the removal of moisture. Such a secondary drying step preferably involves freeze-drying, wherein the additional moisture is removed by sublimation. An alternative type of drying is vacuum drying.

Generally, the secondary drying takes place after the mucoactive has been co-spray dried with a force control agent. In another embodiment, the secondary drying takes place after nebulised mucoactive agent has been spray dried, wherein the mucoactive agent was optionally in a blend with a FCA.

The secondary drying step has two particular advantages. Firstly, it can be selected so as to avoid exposing the heparin to high temperatures for prolonged periods. Furthermore, removal of the residual moisture by secondary drying is significantly cheaper than removing all of the moisture from the particle by spray-drying. Thus, a combination of spray drying and freeze-drying or vacuum drying is economical and efficient.

Secondary drying significantly reduces the moisture content of mucoactive particles (from approximately 8.5% to 2%). This would imply that the mucoactive particles are drying in such a way that there is a hard outer shell holding residual moisture, which is driven off by secondary drying, and entrapped moisture is trapped with in a central core. One could infer that the residence time of the particle in the drying chamber is too short, and that the outer shell is being formed rapidly and is too hard to permit moisture to readily escape during the initial spray drying process.

Secondary drying can also be beneficial to the stability of the product, by bringing down the moisture content of a powder. It also means that drugs which may be very heat sensitive can be spray dried at lower temperatures to protect them, and then subjected to secondary drying to reduce the moisture further, and protect the drug.

In another embodiment of the third aspect of the invention, the moisture profiling involves increasing the moisture content of the spray dried particles.

Preferably, the moisture is added by exposing the particles to a humid atmosphere. The amount of moisture added can be controlled by varying the humidity and/or the length of time for which the particles are exposed to this humidity.

Following spray drying, which also optionally may include secondary drying, it may also be advantageous to mill the powders, for example in an air jet mill, in order to separate any particle agglomerates which have formed strong bridges between particles.

Instead of spray drying the one or more mucoactive agents to form a dry powder formulation, it is also possible to use other methods of preparing a dry powder. For example, many dry powders are formed by micronisation, that is, grinding up larger particles to form small particles of a desired size.

Techniques known as co-milling and mechanofusion, as described in detail in International Publication No. WO 02/43701, produce composite active particles and also are suitable for preparing the dry powder formulations of the present invention.

The composite active particles formed by co-milling and mechanofusion in the present invention are very fine particles of one or more mucoactive agents which have, upon their surfaces, an amount of an FCA. The FCA is preferably in the form of a coating on the surfaces of the particles of one or more mucoactive agents. The coating may be a discontinuous coating. The FCA may be in the form of particles adhering to the surfaces of the particles of one or more mucoactive agents. As explained below, at least some of the composite active particles may be in the form of agglomerates.

When the composite active particles are included in a pharmaceutical composition the FCA promotes the dispersal of the composite active particles on administration

of that composition to a patient, via actuation of an inhaler, as discussed above. Thus, once again, the presence of the FCA is able to increase the FPF and FPD of the dry powder formulation.

It has also been found that the milling of the particles of one or more mucoactive agents in the presence of an FCA produces significantly smaller particles and/or requires less time and less energy than the equivalent process carried out in the absence of the FCA. This allows composite active particles to be produced which have a mass median aerodynamic diameter (MMAD) or a volume median diameter (VMD) of less than 5, 4, 3 or 2μm. It is often much easier to obtain small particles by this method than by other milling methods.

It is known that a milling process will tend to generate and increase the level of amorphous material on the surfaces of the milled particles thereby making them more cohesive. In contrast, the composite particles of the invention will often be found to be less cohesive after the milling treatment.

The word "milling" as used herein refers to any mechanical process which applies sufficient force to the particles of active material that it is capable of breaking coarse particles (for example, particles of mass medium aerodynamic diameter greater than 100μm) down to fine particles of mass median aerodynamic diameter not more than 50μm or which applies a relatively controlled compressive force as described below in relation to the MechanoFusion and Cyclomix methods.

A high degree of force is required to separate the individual particles of one or more mucoactive agents (which tend to agglomerate, especially if they include heparin which is sticky) such that effective mixing and effective application of the FCA to the surfaces of those particles is achieved. It is believed that an especially desirable aspect of the milling process is that the FCA may become deformed in the milling and may be smeared over or fused to the surfaces of the mucoactive particles. It should be understood, however, that in the case where the particles of one or more mucoactive agents are already fine, for example, having an MMAD below 20μm prior to the milling step, the size of those particles may not be significantly reduced.

The important thing is that the milling process applies a sufficiently high degree of force or energy to the particles.

The method generally involves bringing the particles of FCA into close contact with the surfaces of the mucoactive particles in order to achieve coated particles. A degree of intensive mixing is required to ensure a sufficient break-up of agglomerates of both constituents, dispersal and even distribution of FCA over the mucoactive particles.

As a consequence of the milling step, complete or partial, continuous or discontinuous, porous or non-porous coatings may be formed. The coatings originate from a combination of heparin and FCA particles. They are not coatings such as those formed by wet processes that require dissolution of one or both components. In general, such wet coating processes are likely to be more costly and more time consuming than the milling process of the invention and also suffer from the disadvantage that it is less easy to control the location and structure of the coating.

A wide range of milling devices and conditions are suitable for use in the method of the invention. The milling conditions, for example, intensity of milling and duration, should be selected to provide the required degree of force.

Ball milling is a suitable milling method. Centrifugal and planetary ball milling are especially preferred methods. Alternatively, a high pressure homogeniser may be used in which a fluid containing the particles is forced through a valve at high pressure producing conditions of high shear and turbulence.

Shear forces on the particles, impacts between the particles and machine surfaces or other particles and cavitation due to acceleration of the fluid may all contribute to the fracture of the particles and may also provide a compressive force.

Such homogenisers may be more suitable than ball mills for use in large scale preparations of the composite active particles.

Suitable homogensiers include EmulsiFlex high pressure homogenisers which are capable of pressures up to 4000 Bar, Niro Soavi high pressure homogenisers (capable of pressures up to 2000 Bar), and Microfluidics Microfluidisers (maximum pressure 2750 Bar). The milling step may, alternatively, involve a high energy media mill or an agitator bead mill, for example, the Netzch high energy media mill, or the DYNO-mill (Willy A. Bachofen AG, Switzerland). Alternatively the milling may be a dry coating high energy process such as a MechanoFusion system (Hosokawa Micron Ltd) or a Hybridizer (Nara).

Other possible milling devices include air jet mills, pin mills, hammer mills, knife mills, ultracentrifugal mills and pestle and mortar mills.

Especially preferred methods are those involving the MechanoFusion, Hybridiser and Cyclomix instruments. Also especially preferred is an air jet mill.

Other suitable methods include ball and high energy media mills which are also capable of providing the desired high shear force and compressive stresses between surfaces, although as the clearance gap is not controlled, the coating process may be less well controlled than for MechanoFusion milling and some problems such as a degree of undesired re-agglomeration may occur. These media mills may be rotational, vibrational, agitational, centrifugal or planetary in nature.

It has been observed in some cases that when ball milling mucoactive particles with additive material, a fine powder is not produced. Instead the powder was compacted on the walls of the mill by the action of the mill. That has inhibited the milling action and prevented the preparation of the composite active particles. That problem occurred particularly when certain additive materials were used, in cases where the additive material was present in small proportions (typically < 2%), in cases where the milling balls were relatively small (typically < 3mm), in cases where the milling speed was too slow and where the starting particles were too fine. To prevent this occurring it is advantageous to ball mill in a liquid medium. The liquid

medium reduces the tendency to compaction, assists the dispersal of additive material and improves any milling action.

The liquid medium may be high or low volatility and of any solid content as long as it does not dissolve the mucoactive particles to any significant degree and its viscosity is not so high that it prevents effective milling. The liquid medium preferably is not aqueous. The liquid is preferably one in which the additive material is substantially insoluble but some degree of solubility may be acceptable as long as there is sufficient additive material present that undissolved particles of additive material remain. Suitable liquid media include diethylether, acetone, cyclohexane, ethanol, isopropanol or dichloromethane. Liquid media are preferred which are non-flammable, for example dichloromethane and fluorinated hydrocarbons, especially fluorinated hydrocarbons which are suitable for use as propellants in inhalers.

The results of spray drying heparin and jet milling heparin with an FCA are set out below.

Heparin and leucine (95:5) in a 2%(w/w) solution was spray dried using an SL10 spray drier, with a conventional two fluid atomiser. The powder was spray dried at a temperature of 250°C and a nozzle air pressure of 80psi. The liquid flow rate used was 32 ml/min.

The resulting powder was collected in a cyclone. This powder was then secondary dried under vacuum. The powder was then filled into capsules at 20mg, and then fired from a monohaler into a twin stage impinger. The resulting FPF(MD) was 37%. The FPF(MD) increased to 40% following a subsequent air jet milling of the powder to reduce any solid bridges between particles in agglomerates. The powder was also analysed by Malvern particle sizer, and the results are summarised in the table below

The combination of heparin and leucine (95:5) was also air jet milled using a Hosokawa Micron AS50 mill. The material was passed twice through the mill. The

powder was also analysed by Malvern particle sizer, and the results are summarised in the table below.

The D50 value and the FPF(MD) were similar to the results achieved in the above spray dried powders.

Pure heparin powder was air jet milled with two passes and gave an FPF(MD) of only 7%. The D50 of this powder is substantially larger than that of the leucine containing air jet milled sample.

| Sample heparin/leucine (95:5) | D10 | D50 | D60 | D90 | Yield % | FPD<5um |
|---|---|---|---|---|---|---|
| Spray dried collected from cyclone | 1.2 | 2.7 | 3.3 | 7.2 | 55 | 37.4 |
| Spray dried collected from cyclone | 0.4 | 0.9 | 1.1 | >100 | 15 | 25.0 |
| Spray dried collected from cyclone and then jet milled | 1.1 | 2.6 | 3.1 | 5.9 | | 40.2 |
| | | | | | | |
| Spray dried 6.5 hr | | | | | 75 | |
| | | | | | | |
| Jet milled 1x | 0.85 | 3.4 | 4.2 | 8.8 | | 20.4 |
| Jet milled 2x | 0.95 | 3.5 | 4.1 | 7 | | 37.1 |
| Jet milled 3x | 1.1 | 2.8 | 3.3 | 5.5 | | 41.0 |
| | | | | | | |
| Jet milled heparin 2x | | | | | | 7.0 |
| Pure heparin | | | | | | 12.0 |

Clearly there are other known techniques for forming fine particles comprising a mucoactive agent and a FCA. Such techniques include, for example, techniques using supercritical fluids (SCFs), which have been explored for many years for particle production purposes. Similar to the spray-drying technique, this technique provides a direct formation of micron-sized particles suitable for inhalation powders. The most commonly used supercritical fluid technologies for particle production are rapid expansion of supercritical solutions (RESS) and supercritical antisolvent (SAS) or gas antisolvent (GAS) methods.

RESS is based on a rapid expansion of a SCF. The process involves dissolving the drug mixture in a SCF, followed by a rapid expansion of the fluid causing the

compound to precipitate. This technique is capable of producing uniform particles, with control on the size distribution and morphology of particles. However, this technique is limited by the fact that most drugs have low solubility in the SCFs.

SAS is a recrystallisation process that relies on the capability of SCFs to act as an antisolvent to precipitate particles within a liquid solution. Unlike in the RESS technique, SAS does not require a high solubility of the drug compounds in the SCFs. Therefore, SAS is more commercially viable for powder production.

Recently a solution enhanced dispersion by supercritical fluids (SEDS) was introduced, see for example patent publications GB 2322326, WO 95/01324, WO 95/01221, US 5,851,453 and WO 96/00610. This technique is based on simultaneous dispersion, solvent extraction and particle formation in a highly turbulent flow. SEDS is capable of generating uncharged and crystalline product, with the capability of controlling particle size and size distribution by manipulating process conditions.

Another approach is the technique known as emulsion precipitation. This method can be used to prepare fine particles of mucoactive agent and one or more FCAs.

The dry powder compositions of the present invention are preferably delivered by an inhaler device, most preferably by a dry powder inhaler (DPI). This type of inhaler is commonly used for pulmonary administration of a dry powder formulation. Thus, according to a further aspect of the invention, a DPI is provided, for dispensing the composition of the present invention.

The DPI may include a reservoir for holding the powder formulation and a metering mechanism for metering out individual doses of the formulation from the reservoir. Examples of such devices include Turbohaler (trademark) (AstraZeneca) or Clickhaler (trademark) (Innovata Biomed Ltd).

Alternatively, the dry powder inhaler may be arranged to use pre-metered doses of the formulation packaged, for example, in hard or soft gelatin capsules or blister

packs. The Rotahaler (trademark) (GlaxoSmithKline), Spinhaler (trademark) (Rhône-Poulenc Rorer), Cyclohaler (trademark) (Pharmachemie B.V.) and Monohaler (trademark) (Miat) are examples of this type of dry powder inhaler. The invention also provides a metered dose of the formulation contained, for example, in a hard or soft gelatin capsule or blister pack. The aforementioned devices are passive devices, but active devices, such as an Aspirair (trademark) device (see WO 01/00262 and GB 2353222) may also be used.

Preferably, the inhaler is arranged to dispense one or more doses of the formulation, each dose comprising an effective amount of one or more mucoactive agents to be made available for inhalation. The dose may comprise not more than 100mg of one or more mucoactive agents, preferably not more than 50mg, more preferably not more than 25mg and most preferably not more than 20mg of one or more mucoactive agents. The dose may comprise at least 20mg of one or more mucoactive agents, preferably at least 50mg. A preferred dose comprises 70-80mg one or more mucoactive agents.

In another embodiment of the present invention, the DPI is adapted to deliver one or more mucoactive agents to the deep lung of a patient at a dose of at least 5,000 IU.

According to another aspect of the present invention, a package is provided for use in a DPI containing as amount of the composition which comprises at least 20mg of one or more mucoactive agents. Preferably, the DPI according to the invention is arranged to use a package according to the invention.

According to a yet further aspect of the present invention, the compositions according to the invention are used for use in therapy. Preferably, they are for use in treating pulmonary diseases which involve excess mucus in the airways or problems clearing mucus from the airways, examples of which are discussed above.

## Claims

1. A composition for assisting mucus clearance, the composition comprising one or more mucoactive agents for reducing cross-linking within the mucus; for diluting the mucus; for digesting naked DNA and cell debris within the mucus.

2. A composition as claimed in claim 1, wherein the one or more mucoactive agent is able to reduce inflammation.

3. A composition as claimed in claim 1 or 2, comprising two or more mucoactive agents.

4. A composition as claimed in any one of the preceding claims, wherein the mucoactive agent or agents reduce cross-linking within the mucus and dilute the mucus.

5. A composition as claimed in any one of the preceding claims, comprising one or more glycosaminoglycans.

6. A composition as claimed in claim 5, wherein the glycosaminoglycan is heparin and/or a heparinoid.

7. A composition as claimed in claim 6, wherein the heparinoid is danaparoid sodium, or dermatan sulphate.

8. A composition as claimed in claim 6, wherein the heparinoid contains heparin, dermatan sulphate and chondroitin sulphate.

9. A composition as claimed in any one of the preceding claims, comprising sulfated glucosaminoglycans, glycosaminoglycan polysulphate compounds, or sulfated mucopolysaccharides.

10. A composition as claimed in any one of the preceding claims, comprising a monosaccharide, a disaccharide and/or an oligosaccharide.

11. A composition as claimed in any one of the preceding claims, comprising dextran, dextrin, glucose and/or mannitol.

12. A composition as claimed in any one of the preceding claims, comprising an amino acid.

13. A composition as claimed in any one of the preceding claims, comprising rhDNase, gelsolin or thymosin ß4.

14. A composition as claimed in any one of the preceding claims, wherein the composition is a dry powder for pulmonary inhalation.

15. A composition as claimed in claim 14, wherein the composition has a fine particle fraction (<5μm) of at least 50%, and preferably between 70 and 99% or between 80 and 99%.

16. A composition as claimed in claim 14 or claim 15, wherein the composition has a fine particle dose of between 50 and 90%, and preferably between 60 and 70%.

17. A composition as claimed in any one of claims 14-16, comprising particles of at least one mucoactive agent and a force control agent.

18. A composition as claimed in claim 17, wherein the force control agent is an amino acid or peptide, or derivatives thereof, a phospholipid or a metal stearate.

19. A composition as claimed in claim 18, wherein the force control agent is leucine, lysine, cysteine, or mixtures thereof.

20. A composition as claimed in claim 17, wherein the force control agent is included in an amount of up to 50% w/w, preferably less than 10% w/w, and more preferably less than 5% w/w.

21. A composition as claimed in any of claims 14-20, wherein the composition comprises heparin particles having a MMAD of less than 10μm.

22. A composition as claimed in claim 21, wherein the heparin particles have a MMAD of 2-5μm.

23. A composition as claimed in any one of claims 14-22, wherein the composition further comprises carrier particles, preferably wherein the carrier particles have a particle size of at least 20μm.

24. A pharmaceutical composition as claimed in any one of claims 1-23, for use in therapy.

25. A pharmaceutical composition as claimed in claim 24, for treating a pulmonary disease.

26. A pharmaceutical composition as claimed in claim 25, wherein the pulmonary disease involves hypersecretion of mucus or abnormal viscoelasticity of mucus.

27. A pharmaceutical composition as claimed in either of claims 25 or 26, wherein the pulmonary disease is chronic bronchitis, acute asthma, cystic fibrosis (CF), chronic obstructive pulmonary disease (COPD) or bronchiectasis.

28. A method of treating a pulmonary disease comprising the administration of a therapeutically effective amount of a pharmaceutical composition as claimed in any one of claims 1-23 to a subject in need of such treatment.

# Abstract


## Pharmaceutical Compositions

The present invention relates to pharmaceutical compositions which are useful in the treatment of diseases where excess mucus is present in the respiratory tract, such as cystic fibrosis and chronic obstructive pulmonary disease. In particular, the invention relates to pharmaceutical compositions for administration by pulmonary inhalation.